6.15 Enzyme-Linked Immunosorbent Assay (ELISA)

The following section shall be used to ensure that the Tecan EVO 75 instruments used in the Toxicology Unit for Enzyme-Linked Immunosorbent Assay (ELISA) are properly maintained for accurate presumptive identification of opiates, buprenorphine, cannabinoids, cocaine/benzoylecgonine, barbiturates, benzodiazepines and other validated assays in submitted evidence.

6.15.1 Instrument Start Up

Before each run, the scientist/technician shall:

- Check and/or fill water containers.
- Check and/or empty waste containers.
- Turn on the Tecan EVO 75 instrument platform.
- Take the HydroFlex Washer out of Night Rinse mode.
- Turn on the Sunrise Reader.
- Perform a Flush and Prime of the system, checking for leaks and/or air bubbles in the system tubing and syringes.
- Visually inspect the Teflon coated pipettes, reagent troughs, sample racks, and assay plate platforms for residue, dust, and damage. If needed, clean with a lint-free cloth and isopropyl alcohol or replace.

6.15.1.1 Calibration

The ELISA method of testing is a preliminary drug screen. Therefore, results for case samples are report as positive or negative based on the difference data absorption rates obtained from case samples as compared to the cutoff values, established by the positive control. All positive results will be confirmed with appropriate testing.

6.15.1.1.1 Reagents

Check conjugant and reagent levels and manufacturer expiration dates; fill or replace if needed. Any new plate, conjugant or reagent opened, discarded or used up shall be recorded in the ELISA logbook and on the ELISA login sheet included with every QC packet. Reagents used are as follows:

- Opiates Direct ELISA Kit
- Buprenorphine Direct ELISA Kit
- Cannabinoids (THCA/CTHC) Direct ELISA Kit
- Cocaine/Benzoylecgonine Direct ELISA Kit
- Barbiturate Direct ELISA Kit
- Benzodiazepines ELISA Kit
- Any other validated ELISA kits

6.15.1.1.2 Controls

ELISA positive and negative controls are made within the laboratory. Therefore, every time new controls are made they shall be analyzed concurrently with the controls previously verified and the difference data means compared. The difference data mean for each assay for the new working reference control standard should be within +/- 20% of the difference data mean of the corresponding assay for the working reference control standard currently in use. The difference data mean values for the positive control for each assay shall be documented in the appropriate ELISA instrument record book each time new working reference control standards are made.

If the new working reference control standard is slightly outside of the +/- 20% acceptance criteria, then the scientist/technician shall make a low positive control (50% below the concentration of the control standard concentration) and a high positive control (100% above the concentration of the control standard concentration. If the ranges of the low positive control, positive control and high positive control (difference data mean +/- 2 standard deviations for each) do not overlap, then the new working reference control standard will be considered fit for use. See ELISA Validation notebook for examples.

6.15.2 Data Evaluation

6.15.2.1 Instrument operation shall be verified by analyzing known positive and negative controls made by the examiner/technician with each batch of unknown case samples. The difference data mean values for the positive control for each assay shall be documented in the appropriate ELISA instrument record book each time case samples are analyzed.

6.15.2.2 The positive control will be analyzed in duplicate at the beginning of the run (positions A1 and B1 on the well plate). The negative control will be analyzed in duplicate immediately following the positive control (positions C1 and D1). The difference data mean of the positive control must be less than the difference data mean for the negative control in order for results to be reported (PC<NC).

6.15.2.3 The difference data variation coefficient for both the positive and negative controls shall not exceed 20%.

6.15.2.4 If the Sunrise Reader is unable to obtain results (i.e. difference data reported as “No Calc”), these samples shall be pipetted with the next batch of cases and reanalyzed, provided there is sufficient sample for analysis.

6.15.3 Maintenance

6.15.3.1 If the control results are outside the acceptance criteria, the cause will be assessed and corrected. If the problem cannot be resolved the instrument shall be marked “out of service” until the issue is resolved.

6.15.3.2 The Hydroflex Washer should be placed in Night Rinse mode once each normal business day to ensure that the washer is functioning properly.

6.15.3.3 An acid/base wash shall be performed monthly and dates performed/expire shall be documented.

6.15.3.4 All maintenance or repairs shall be recorded in the appropriate ELISA instrument logbook and include at a minimum: the date, initials and type of maintenance or repair performed.

6.15.3.5 Positive and negative controls shall be analyzed concurrently with case samples, following any maintenance or repair.

6.15.3.6 Analyzed data shall be saved in the Magellan data analysis software. Hard copies shall be maintained in the case file.

6.15.4 Instrument Shut Down

After each run, the examiner shall:

- Empty all troughs into the appropriate storage containers.
- Close/cover and put away all reagents and controls in the proper storage locations (refrigeration or room temperature).
- Discard used well strips, as well as all culture tubes containing aliquots of samples.
- Visually inspect the Teflon coated pipettes, reagent troughs, sample racks, and assay plate platforms for residue, dust, and damage. If needed, clean with a lint-free cloth and isopropyl alcohol or replace.
- Check and/or fill water containers.
- Check and/or empty waste containers.
- Put the HydroFlex Washer in Night Rinse mode.
- Submit the ELISA quality control packet for review. This packet should include the following:
    - ELISA log in sheet
    - Instrument run list from Navitrak
    - Sample pipette run list
    - Sample pipetting checklist
    - Data printouts from Magellan for each assay analyzed

6.15.5 References

Magellan Reference Guide, Part no. 30066407, Revision1.3, Tecan Austria GmbH, Austria, 2013

Product Insert for the Direct ELISA Kits, Immunalysis Corporation, 2001.

Tecan Freedon EVO 75 Operating Manual, ID: 393248, Tecan Schweiz AG, Switzerland, 2013.

Tecan HydroFlex Washer Instructions for Use, Part no. 30086671, Revision 1.4, Tecan Austria GmbH, Austria, 2013.

Tecan Sunrise Microplate Absorbance Reader Instructions for Use, Part no. 30086638, Revision 2.3, Tecan Austria GmbH, Austria, 2013.